On Searching In, Sampling Of, and Dynamically Moving Through Conformational Space of Biomolecular Systems: A Review

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Abstract: Methods to search for low-energy conformations, to generate a Boltzmann-weighted ensemble of configurations, or to generate classical-dynamical trajectories for molecular systems in the condensed liquid phase are briefly reviewed with an eye to application to biomolecular systems. After having chosen the degrees of freedom and method to generate molecular configurations, the efficiency of the search or sampling can be enhanced in various ways: (i) efficient calculation of the energy function and forces, (ii) application of a plethora of search enhancement techniques, (iii) use of a biasing potential energy term, and (iv) guiding the sampling using a reaction or transition pathway. The overview of the available methods should help the reader to choose the combination that is most suitable for the biomolecular system, degrees of freedom, interaction function, and molecular or thermodynamic properties of interest.

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Introduction

Computer modeling of (bio)molecular systems has become a standard technique to study and describe the properties and behaviour of such systems in terms of interactions between atoms or electrons of atoms. Although quantum mechanics governs these interactions, it can only be used to model a very limited number of degrees of freedom of a biomolecular system because of the complexity of the algorithms to solve the (time-dependent) Schrödinger equation. Leaving processes such as electron or proton transfer or processes that involve making or breaking of covalent bonds aside, classical mechanics offers a good approximation of quantum mechanics, e.g. for processes such as polypeptide folding, molecular complexation, partitioning of molecules between different environments, and the formation of molecular aggregates (e.g. membranes) out of mixtures. Here we only consider molecular systems that are described in terms of a classical Hamiltonian

$$\mathcal{H}(\mathbf{p},\mathbf{q}) = \mathcal{K}(\mathbf{p},\mathbf{q}) + \mathcal{V}(\mathbf{p},\mathbf{q}), \tag{1}$$

which depends on the $\mathbf{q} \equiv (\mathbf{q_1}, \mathbf{q_2}, \ldots, \mathbf{q}_{\mathbf{N_{df}}})$ generalized coordinates and $\mathbf{p} \equiv (\mathbf{p_1}, \mathbf{p_2}, \ldots, \mathbf{p}_{\mathbf{N_{df}}})$ conjugate momenta of the chosen N_{df} degrees of freedom. The kinetic energy term is denoted by $\mathcal{K}(\mathbf{p},\mathbf{q})$ and the potential energy one by $\mathcal{V}(\mathbf{p},\mathbf{q})$. The classical-mechanical equations of motion are then

$$\begin{aligned} \frac{d}{dt}\mathbf{q_i} &= \frac{\partial \mathcal{H}(\mathbf{p},\mathbf{q})}{\partial \mathbf{p_i}} \quad i = 1, 2, \ldots, N_{\mathrm{df}}, \\ \frac{d}{dt}\mathbf{p_i} &= -\frac{\partial \mathcal{H}(\mathbf{p},\mathbf{q})}{\partial \mathbf{q_i}} \quad i = 1, 2, \ldots, N_{\mathrm{df}}. \end{aligned} \tag{2}$$

When using Cartesian coordinates, $q \equiv x$, and assuming that the potential energy is independent of the momenta, one has

$$\mathcal{K}(\mathbf{p},\mathbf{x}) = \sum_{\mathbf{i=1}}^{\mathbf{N_{df}}} \frac{\mathbf{p_i^2}}{2m_i}, \tag{3}$$

and Eq. (2) reduce to Newton's equations of motion

$$m_i \frac{d^2 x_i}{dt^2} = \frac{\partial}{\partial x_i} \mathcal{V}(x_1, x_2, \ldots, x_{N_{\mathrm{df}}}), \tag{4}$$

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where we have indicated the mass governing the motion of the i-th degree of freedom by m_i. The interaction function

$$\mathcal{V}(\mathbf{x}) \equiv \mathcal{V}(x_1, x_2, \ldots, x_{N_{\mathrm{df}}}) \tag{5}$$

is an effective interaction: it describes the interaction between explicitly treated degrees of freedom averaged over the omitted atomic or electronic degrees of freedom.

Because biomolecular modeling involves microscopic systems at non-zero temperatures T, the basic theory to describe such a system is quantum or classical statistical mechanics. Consequently, the state of a biomolecular system is characterised by a statistical–mechanical ensemble of configurations. At fixed particle number, volume, and temperature this is a canonical ensemble, in which the weight of a molecular or system configuration is given by the Boltzmann factor

$$e^{-\mathcal{V}(\mathbf{x})/k_{\mathrm{B}}T}, \tag{6}$$

where k_{B} denotes Boltzmann's constant. This implies that the equilibrium properties of the system are determined by those parts of configuration space, for which $\mathcal{V}(\mathbf{x})$ is minimal. Therefore, one of the basic challenges to biomolecular modeling is to develop methodology to efficiently search the biomolecular energy surface $\mathcal{V}(\mathbf{x})$ for regions of low energy. The statistical–mechanical nature of this search problem implies that it cannot be reduced to the problem of finding the global (energy) minimum of the multi-dimensional function $\mathcal{V}(\mathbf{x})$. Statistical–mechanically the free energy

$$F = U - TS, \tag{7}$$

composed of an energetic contribution U and an entropic contribution $-TS$, is minimal, not the energy U. The entropy is a measure of the extent of configurational space ($\mathbf{x}$) accessible to the molecular system at a given temperature T. Figure 1 illustrates that lowest energy does not necessarily mean lowest free energy. Two parts of configurational space $\mathbf{x_1}$ and $\mathbf{x_2}$ may have $U(\mathbf{x}_1) \ll U(\mathbf{x_2})$, whereas $F(\mathbf{x}_1) > F(\mathbf{x_2})$ due to $S(\mathbf{x}_1) \ll S(\mathbf{x_2})$ at the given temperature T. This means that searching for and finding the global energy minimum for a biomolecular system is meaningless when its entropy accounts for a sizable fraction of its free energy.

When considering methods to generate molecular configurations, we distinguish three types based on the characteristics of the set of generated configurations:

1. Methods that generate a series of nonrelated low-energy configurations.
2. Methods that generate a properly (Boltzmann) weighted set of configurations.
3. Methods that generate a (classical) dynamical trajectory of configurations, which are moreover properly (Boltzmann) weighted.

Methods of type 1 should only be used for zero entropy systems, whereas methods of types 2 and 3 yield proper ensembles, so can be used to compute thermodynamic and other equilibrium properties. Only methods of type 3 yield information on dynamical properties of the system.

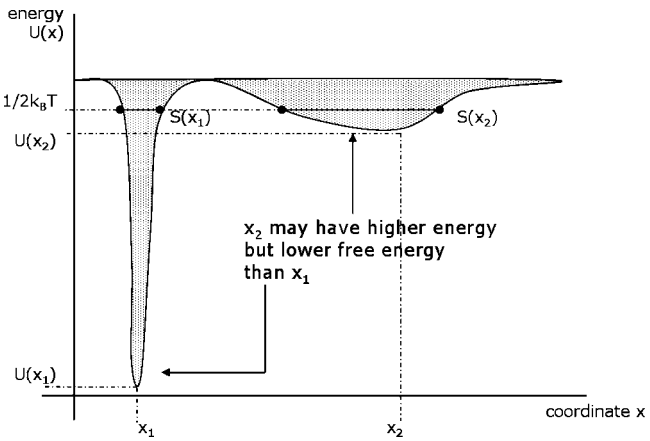

Figure 1. Energy (U)–entropy (S) compensation at finite temperature T.

Generally, modeling of a molecular system involves four choices.

1. Which degrees of freedom are explicitly modeled, i.e. treated in expressions (5) and (6).
2. Which interaction function or force field $\mathcal{V}(\mathbf{x})$ is used to calculate the potential energy of the system and the forces along the explicitly treated degrees of freedom.
3. Which algorithm is used to search for those parts of configurational space for which $\mathcal{V}(\mathbf{x})$ is minimal (type 1), or to sample (type 2) or simulate (type 3) the motion along the degrees of freedom.
4. How are the spatial boundaries of the system modeled and which thermodynamic boundary conditions are used.

Biomolecular systems have a density comparable to solids or liquids, but lack the symmetry ordering of the former. They constitute a many-particle system for which no simple reduction to a few degrees of freedom is possible: one is faced with an essential many-particle problem, the solution of which can only be adequately described by numerical simulation. The four choices mentioned all have an impact on the accuracy and efficiency of the modeling. It is the purpose of this article to consider the choices to be made from the view-point of accurately and efficiently generating low-energy configurations or ensembles or trajectories for biomolecular systems. Because of the great variety in methods and applications in the literature, we only classify and mention the available methods, with references, and do not review their applications. The classification given may help the reader to find his or her way in the jungle of methods and to choose a combination of methods and techniques that suits his or her purpose best.

Choice of Degrees of Freedom

Generally, biomolecular systems are composed of more atoms than can be reasonably modeled on a computer. Depending on the property of interest, the essential degrees of freedom are to be identified and explicitly treated. The remaining ones are then omitted and

their effect upon the interaction between or along the explicitly treated ones is included in an averaged manner in the function $\mathcal{V}(\mathbf{x})$. For example, in structure refinement of proteins, solvent degrees of freedom are generally omitted,[1,2] although it is experimentally known that protein structure is sensitive to solvent composition. Polypeptide folding or protein–ligand complexation is sometimes modeled without explicitly treating solvent degrees of freedom.[3,4] This enables fast simulation, because the number of solvent degrees of freedom is generally much larger than that of the solutes, but limits the accuracy[5] and applicability of such implicit solvation models. For example, complex enthalpy–entropy compensation effects cannot be captured.[6]

On the other hand, implicit treatment of aliphatic hydrogen atoms by using united $-CH_n-$ ($n = 1, 2$) and $-CH_3$ atoms in simulation of systems that contain many of such moieties, like lipid mixtures and membranes, saves easily a factor of four to nine in computing effort, which is dominated by the computation of non-bonded forces. Yet, no loss in accuracy is observed when comparing properties calculated using all-atom versus united-atom models.[7,8] The use of united atoms is an example of the technique of coarse-graining: groups of atoms, molecules or fragments of molecules are treated as single particles or beads, whose motion is simulated using a single coarse-grained (CG) force field describing inter-bead interactions. When the energy function $\mathcal{V}(\mathbf{x})$ of such a CG model is chosen to be smooth and short-ranged, the efficiency of CG simulations can be orders of magnitude higher than the corresponding fine-grained (FG) simulations, be it at the expense of the loss of atomic detail and some accuracy.[9–13] Recently, it has been proposed to combine FG and CG models in one simulation, while the contribution of the two grain levels to the interaction between the atoms or beads is governed by a grain level parameter λ. This allows for a continuous switching between grain levels, which can in turn be exploited in the replica-exchange technique to enhance the sampling at the various λ-values.[14,15]

The performance of a CG model in practical applications depends on the chosen coarse-graining procedure: (i) the model resolution (how many FG particles are mapped onto one CG bead), (ii) the mapping procedure (how the CG bead positions are defined in terms of the FG atom positions), (iii) the form of the energy function $\mathcal{V}(\mathbf{x})$ of the CG Hamiltonian, and (iv) the experimental and/or FG simulation properties against which the CG model parameters were optimised.

The number of degrees of freedom to be simulated can also be reduced by constraining those which are characterised by high-frequency motions that are not influencing the properties of interest. For molecular systems one may think of bond-length and bond-angle degrees of freedom.[16] Holonomic (time-independent) constraints can be implemented in two ways: (i) by formulating Lagrange equations of motion in generalized (e.g. torsional angle coordinates),[17,18] or (ii) by formulating these equations in Cartesian coordinates (e.g. using Newton's eq. (4)) and then using Lagrange multipliers to satisfy the constraints for each configuration generated.[19,20] When considering branched polymers, the choice of internal coordinates (bond lengths, bond angles, and torsional angles) to serve as generalized coordinates seems to be natural, because they allow for constraining bond lengths and angles by simply omitting them from the equations of motion. However, the equations of classical dynamics (2) expressed in internal, generalised coordinates $q \equiv \theta$

$$\sum_{j=1}^{N_{\mathrm{df}}} a_{ij} \frac{d^2\theta_j}{dt^2} = -\frac{\partial}{\partial\theta_i}\mathcal{V}(\theta_i, \theta_2, \ldots, \theta_{N_{\mathrm{df}}}) - \sum_{j=1}^{N_{\mathrm{df}}} b_{ij} \left(\frac{d}{dt}\theta_j\right)^2 - \sum_{j=1}^{N_{\mathrm{df}}}\sum_{k=1}^{N_{\mathrm{df}}} c_{ijk} \left(\frac{d}{dt}\theta_j\right)\left(\frac{d}{dt}\theta_k\right), \quad i = 1, 2, \ldots, N_{\mathrm{df}} \qquad (8)$$

are considerably more complex than when expressed in Cartesian coordinates, Eq. (4). They contain two additional summations over the number of degrees of freedom and two additional quadratic (i.e. non-linear) terms in the generalised velocities. Eq. (8) has been presented in different forms,[1,2,17,18,21–25] and the coefficients a_{ij}, b_{ij}, and c_{ijk} depend on the atomic masses and the molecular topology of the polymer considered. Simulation of a protein through Eq. (8) requires a much larger computational effort than simulation through Eq. (4), since at each time step a set of N_{df} non-linear equations is to be solved. One iteration to this end may take as much computational effort as the calculation of all forces and energies, thereby doubling the overall computational expense.[2] Therefore, the use of Cartesian coordinates, i.e. Newton's equations of motion in combination with Lagrange multipliers to impose constraints is recommended.[26]

An extension of the concept of a (hard) constraint is a flexible, or soft or adiabatic constraint, in which the length of a constrained distance is not a constant through the simulation, but varies per time step without involving kinetic energy.[27–29] This eliminates the high-frequency motions in the system, while keeping the constrained degrees of freedom flexible.

The number of degrees of freedom can also be kept low by choosing appropriate periodic boundary conditions. When simulating a spherical solute, use of a more spherically shaped configurational periodic box instead of the standardly used cubic or rectangular periodic box may considerably reduce the number of solvent molecules needed to fill the space left after insertion of the solute in the box. For a spherical solute the number of atomic degrees of freedom can be reduced by at least one quarter in this way.[30]

Types of Methods to Search, Sample, or Dynamically Move Through Configuration Space

A variety of search, sampling, or simulation methods is available, each with its particular strengths and weaknesses, depending on (i) the form of the function $\mathcal{V}(\mathbf{x})$ and (ii) the number and types of degrees of freedom of the system. These methods are based on the use of molecular coordinates $\mathbf{q}$ or $\mathbf{x}$ as variables. For methods that use as variables other quantities than molecular coordinates we refer to Section VIII. Two basic types of methods can be distinguished, systematic search and heuristic search.

Systematic or exhaustive search methods scan the complete or a significant fraction of the configuration space of the molecular system. Particular subspaces can be excluded from the search without loss in the quality of the solution found, thanks to rigorous arguments that these subspaces cannot contain the desired solution.[31] Such arguments are based on *a priori* knowledge, often of physical

or chemical nature, about the structure of the space or energy function or hypersurface to be searched. Systematic search techniques can only be applied to small molecules involving only a few degrees of freedom,[32–36] because of the exponential growth of the required computing effort as function of the number of degrees of freedom included in the search.

Heuristic search methods, although visiting a tiny fraction of the configuration space, aim at generating a possibly representative (in the Boltzmann weighted sense) set of system configurations. These methods may generally be divided into two or three types.

1. Non-step methods, in which a series of system configurations is generated, which are independent of each other. One example is the so-called distance geometry metric matrix method,[37,38] which, for a search problem that can be cast into a distance based form, generates, at least in principle, an uncorrelated series of random configurations. Another example is based on the technique of threading,[39,40] in which linear combinations of parts of protein structures as obtained from a protein structure data bank are used to generate novel possible protein structures.[41]
2. Step methods that build a complete molecular or system configuration from configurations of fragments of the molecule or system in a stepwise manner. Examples are the build-up procedure of Scheraga,[42,43] combinatorial build-up methods that make use of dynamic programming techniques[44] and Monte Carlo (MC) chain growing methods,[45,46] such as the so-called configurational bias Monte Carlo (CBMC) technique.[47]
3. Step methods, such as energy minimisation (EM), Metropolis Monte Carlo (MC), molecular dynamics (MD), and stochastic dynamics (SD),[48] that generate a new configuration of the complete system from the previous configuration. These methods can be classified according to the way in which the step direction and step size are chosen, see Figure 2: (a) according to the energy $\mathcal{V}(\mathbf{x})$, (b) according to the gradient of $\mathcal{V}(\mathbf{x})$, (c) according to the curvature of $\mathcal{V}(\mathbf{x})$, (d) at random, and (e) according to a memory of the path followed so far. Energy minimization can be based on only energy values and random steps (simplex methods), or on energy and energy gradient values (steepest-descent and conjugate-gradient methods), or on second-order derivatives of the energy (Hessean matrix methods). In MC methods the step direction is taken at random, and the step size is limited by the Boltzmann acceptance criterion: when the potential energy of the system changes by $\Delta V < 0$, the step in configuration space is accepted while for $\Delta V > 0$, the step is accepted with probability $\exp(-\Delta V/k_{\mathrm{B}}T)$. In force-biased MC[49] the direction of the force, the negative of the local gradient $\partial V(x)/\partial x$, is also used. In MD simulation the step is determined by the force and by the inertia of the degrees of freedom, which serves as a short-time memory of the path followed so far. In SD simulation a random component is added to the force, the size of which is determined by the temperature of the system and the atomic masses and friction coefficients. In the potential-energy contour tracing (PECT) algorithm[50,51] and in the potential-energy annealing conformational search (PEACS) algorithm,[52] the energy values are monitored and kept constant (PECT) or annealed (PEACS) to locate saddle points and pass over these. The catalytic tempering MC algorithm[53] is based on similar ideas. In MD and SD memory is built into the trajectory through inertial effects. Information on the history of the system can also be included in the force by averaging previous forces.[54–58] There exists a large variety of search procedures based on stepping through configuration space using a combination of the five mentioned basic elements energy, gradient, Hessean, randomness, and memory, combined in one way or the other.[59]

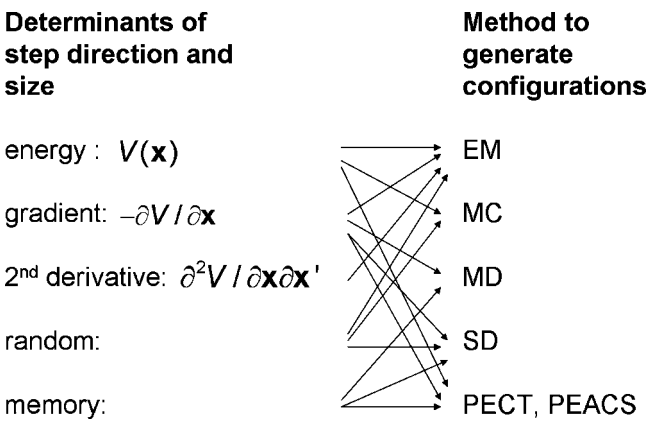

Figure 2. Heuristic methods to search configuration space for configurations $\mathbf{x}$ with low energy $\mathcal{V}(\mathbf{x})$. EM, energy minimisation; MC, Monte Carlo; MD, molecular dynamics; SD, stochastic dynamics; PECT, potential energy contour tracing; PEACS, potential energy annealing conformational search.

The efficiency of search methods for biomolecular systems is severely restricted by the nature of the energy hypersurface $\mathcal{V}(\mathbf{x})$ that is to be explored to find low energy regions. Because of the occurrence of a multitude of high energy barriers between local minima, the radius of convergence of the step methods is generally very small. Therefore, a variety of techniques have been developed to enhance the search and sampling power of searching methods. These are reviewed in Section V.

Techniques to Speed Up a Simulation

For a system containing N atoms, the number of pairwise nonbonded interactions equals $N(N-1)/2$. The computing time for the calculation of all these interactions is proportional to N^2. However, generally not all these interactions need to be computed. Different types of atomic interactions have different spatial ranges. The electrostatic interaction between two charges is proportional to r^{-1}, where r is the distance between the charges. The dipolar interaction is of shorter range, that is, proportional to r^{-3}. The van der Waals interaction is of still shorter range, proportional to r^{-6}, only the first and second neighbor shells contribute significantly to the interaction. In this situation the application of a cut-off radius beyond which no detailed atom–atom interactions are taken into account, but only represented in a mean-field, e.g. a reaction-field, sense, is appropriate. Once the nearest neighbors are found, which is an operation proportional to N,[60–62] the computation of the nonbonded interaction becomes proportional to N as well. Because electrostatic interactions are long-ranged, so-called

particle-particle-particle-mesh techniques have been introduced[62] to compute these efficiently.[63] The computational effort scales with *NlogN* due to the use of fast Fourier transform techniques.[64] An alternative is to approximate the medium beyond a given cut-off distance R_{rf} from a specific atom or molecule by a dielectric continuum of permittivity ϵ_{rf}[65] and ionic strength I_{rf}.[66]

The length of the integration time step Δt is in MD or SD simulation limited by the highest frequency (ν_{max}) motions occurring in the molecular system,

$$\Delta t \ll \nu_{max}^{-1} = \tau_{min}, \tag{9}$$

where $\nu^{-1} \equiv \tau$, an oscillation or relaxation time. For a precise integration of the equations of motion, condition (9) must be satisfied. However, it may be that one is only interested in some average properties of the system. If those are not essentially dependent on ensemble fluctuations, the time step Δt may be lengthened beyond condition (9).

In biomolecular systems three frequency ranges can be distinguished:

1. High-frequency bond-stretching forces $\mathbf{f}^{hf}$ with an approximate oscillation or relaxation time τ^{hf} of about 10 fs,
2. low-frequency long-range Coulomb forces $\mathbf{f}^{lf}$ with $\tau^{lf} \approx 1000$ fs or larger, and
3. the remaining intermediate-frequency forces $\mathbf{f}^{if}$ with $\tau^{if} \approx 40$ fs.

The contribution of these different forces to the atomic trajectories may be integrated using three different time steps, each satisfying condition (9) with the appropriate relaxation time τ. When applied to bond-stretching forces, such a multiple-time-step (MTS) integration scheme[30,67] saves a factor two to three in computing effort.[67–70] When applied to the long-range Coulomb forces, the so-called twin-range method saves about a factor of 5–10 because of the fact that the evaluation of these forces dominates the force calculation.[30,71,72] The same kind of reasoning may also be applied to Monte Carlo simulations.[73,74]

A few other numerical and conceptual tricks that may be used to speed-up a simulation are discussed elsewhere.[75]

Search and Sampling Enhancement Techniques

In Table 1, three general types of search and sampling enhancement techniques are distinguished.

Deformation or Smoothening of the Potential Energy Hypersurface to Reduce Barriers

a. Generally, a smoothening of the potential energy function $\mathcal{V}(\mathbf{x})$ allows for a faster search for its minima. This technique has been applied to different problems, such as structure determination based on X-ray diffraction or NMR spectroscopic data, conformational search, and protein structure prediction. In method Ia of Table 1 the electron density of a biomolecular crystal is smoothened by the omission of high-resolution diffraction intensities when backcalculating the electron density from these through Fourier transforms. This smoothening enhances the radius of convergence of the structure refinement.
b. When building protein structure from atom-atom distance data obtained from NMR, the convergence of the configurational search process is enhanced by gradually introducing distance restraints that connect atoms at longer distance along the polypeptide chain in the potential energy function. This is called a variable-target function method.[76]
c. The hard core of atoms, i.e. the strong repulsive interaction between atoms overlapping with each other, is responsible for many barriers on the energy hypersurface of a molecular system. These barriers can be removed by making the repulsive short-range interactions between atoms soft.[77–80] Soft-core atoms smoothen the energy surface and lead to strongly enhanced sampling.[81]

Table 1. Techniques to Enhance the Searching and Sampling Power of Simulation Methods.

I.	Deformation or smoothening of the potential energy surface
	a. Omission of high-resolution structure factor data in structure refinement based on X-ray diffraction data
	b. Gradual introduction of longer-range distance restraints in variable-target structure refinement based on NMR NOE data
	c. Softening of the hard core of atoms in the non-bonded interaction (soft-core atoms)
	d. Reduction of the ruggedness of the energy surface through a diffusion-equation type of scaling
	e. Avoiding the repeated sampling of an energy well through local potential energy elevation or conformational flooding
	f. Softening of geometric restraints derived from experimental (NMR, X-ray) data through time-averaging of these
	g. Circumvention of energy barriers through an extension of the dimensionality of the Cartesian space (4D-MD)
	h. Freezing of high-frequency degrees of freedom through the use of contraints
	i. Coarse-graining the model by reducation of the number of interaction sites
II.	Scaling of system parameters
	a. Temperature annealing
	b. Tight coupling to heat bath
	c. Mass scaling
	d. Mean-field approaches
III.	Multi-copy searching and sampling
	a. Genetic algorithms
	b. Replica-exchange and multi-canonical algorithms
	c. Cooperative search: SWARM

For details see text of Section V.

d. In the diffusion-equation based deformation methods,[81,82] the deformation of the energy surface driving a simulation is made proportional to the local curvature (second derivative) of the surface, which leads to a preferential smoothening of the sharpest peaks and valleys of the surface and very efficient search. The potential energy surface can be deformed in a great variety of ways. The corresponding search or sampling algorithms sail under an equally wide variety of names: potential-scaled MD,[83] stochastic tunneling,[84,85] q-jumping,[86] and Nose-Hoover deformation.[87]
e. Incorporation of information on the energy hypersurface obtained during the search into the potential energy function is another possibility to enhance sampling. Once a local energy minimum is found, it is removed from the energy surface by a suitable local deformation of the potential energy function. This idea is the basis of "tabu" search,[88,89] the deflation method,[90] and the local-elevation search method,[91] which was recently also called meta-dynamics.[92] The method of conformational flooding[93] is based on the same idea. Other variations can be found as well.[94,95]
f. Another way to introduce a memory into the search is the use of a potential energy term which is a running average over the atomic trajectories or ensemble generated so far, rather than its instantaneous value.[54] Application of this type of time-dependent or ensemble-dependent restraints in protein structure determination based on NMR or X-ray data leads to much enhanced sampling of the molecular configuration space.[96,97]
g. Barriers in the energy hypersurface can be circumvented by an extension of the dimensionality of the configuration space beyond the three Cartesian ones. The technique of energy embedding[98] locates a low-energy conformation in a high-dimensional Cartesian space and gradually projects this conformation to three-dimensional Cartesian space while perturbing its energy and configuration as little as possible. Variations on the original procedure have been proposed.[99–102] Dynamic search methods can also be used in conjunction with an extension of the dimensionality. By performing MD in four-dimensional Cartesian space, energy barriers in three-dimensional space can be circumvented[103] and free energy changes calculated.[104]
h. A long used standard technique to smoothen the energy surface is to freeze the highest-frequency degrees of freedom of a system through the application of constraints.[19,105] Bond-length constraints are standardly applied in biomolecular simulation and allow for a four times longer time step size.[16,105,106] High frequency motion elimination can also be achieved through flexible constraints.[27]
i. A coarse-graining of the molecular model,[9–13] which involves a reduction of the number of interaction sites, generally leads to a smoothening of the energy surface. This may allow the use of simulation time steps that are much (factor of 15) longer than the ones used in fine-grained (atomic) simulations.[107]

Scaling of System Parameters can also be used to Enhance Sampling

a. The technique of simulated temperature annealing[108] involves simulation or search at a high temperature T followed by gradual cooling. By raising the temperature, the system may more easily surmount energy barriers, so a larger part of configurational space can be searched. The technique of simulated temperature annealing has been widely used in combination with MC, MD, and SD simulation. An example of potential energy annealing can be found in ref. 52. The so-called J-walking algorithm[109] also uses temperature variations to enhance the sampling.
b. One way of keeping a constant temperature in a simulation is to use an additional equation that linearly couples the actual temperature $T(t)$ to a reference or heat-bath temperature T_{ref}[110]

$$\frac{d}{dt}T(t) = \tau_T^{-1}(T_{\mathrm{ref}} - T(t)), \tag{10}$$

the coupling strength being determined by the coupling time τ_{T}. When choosing this parameter close to the time step ($\tau_{\mathrm{T}} \geq \Delta t$), the kinetic energy or velocities are enhanced when the system's potential energy increases and the velocities are reduced in low potential energy regions. This enhances the sampling.
c. Scaling of atomic masses can be used to enhance sampling. In the classical partition function and in case no constraints are applied, the integration over the atomic momenta can be carried out analytically, separately from the integration over the coordinates. Thus, the atomic masses do not appear in the configurational integral, which means that the equilibrium (excess) properties of the system are independent of the atomic masses. This freedom can be exploited in different ways to enhance the sampling. By increasing the mass of specific parts of a molecule, their relative inertia is enhanced, which eases the surmounting of energy barriers,[111,112] and may allow for longer time steps. A reduction of the mass of the solvent molecules has been shown to lead to enhanced sampling of the folding/unfolding equilibrium of a polypeptide in explicit solvent simulation.[113] The canonical adiabatically free energy sampling (CAFES) algorithm also exploits inertia to speed up the occurrence of rare events.[114]
d. Enhanced sampling by a mean-field approximation is obtained by separating the biomolecular system into two parts, A and B, each of which moves in the average field of the other. The initial configuration of the system consists of N_{A} identical copies of part A and N_{B} identical copies of part B, where the positions of corresponding atoms in the identical copies may be chosen to be identical. The force on atoms in each copy of part A exerted by the atoms in all copies of part B is scaled by a factor N_{B}^{-1}, to obtain the mean force exerted by part B on the individual atoms of part A. The force on atoms in each copy of part B exerted by the atoms in all copies of part A is scaled by a factor N_{A}^{-1}, to obtain the mean force exerted by part A on the individual atoms of part B. The forces between the different copies of part A are zero, and so are the forces between the different copies of part B. The MD simulation involves the integration of Newton's equation of motion, $\mathbf{f} = m\mathbf{a}$, for all copies of parts A and B simultaneously. Thus one obtains N_{A} individual trajectories of part A in the mean field of part B and vice versa. This comes at the loss of correct dynamics: Newton's third law, $\mathbf{f}_{\mathrm{AB}} = -\mathbf{f}_{\mathrm{BA}}$ is violated. The technique only enhances efficiency when the system is partitioned into parts of very different sizes, e.g. $size(A) \ll size(B)$ and the bigger part is represented by one copy: $N_{\mathrm{B}} = 1$. Locally enhanced searching and sampling (LES) procedures based on

a mean-field approximation have been proposed in different forms.[115–120]

Multi-copy Simulation with a Given Relation Between the Copies can also be used to Enhance Searching and Sampling

In the mean-field approach sketched before multiple copies of a part of the system were simulated. This idea has been used in different ways to enhance searching and sampling, see Table 1.

a. In genetic algorithms[121] a pool of copies of the biomolecular system in different configurations is considered and new configurations are created and existing ones deleted by mutating and combining (parts of) configurations according to a given set of rules.
b. In the so-called replica-exchange algorithm multiple copies of the system are simulated by MC, MD, or SD, each at a distinct temperature. From time to time copies at adjacent temperatures are exchanged using an exchange probability based on the Boltzmann factor in eq. (6). This leads in the limit of infinite sampling to Boltzmann-distributed (canonical) ensembles for each temperature.[122] So-called multi-canonical algorithms are a generalisation of this procedure.[123] This type of algorithm has been used to simulate proteins *in vacuo*.[122] The inclusion of solvent degrees of freedom may impair the efficiency of the algorithm.[124] Dynamical information is lost in the exchanges. A variety of schemes of this type has been recently proposed: generalised-ensemble algorithms,[123,125] local and partial replica-exchange,[126] parallel replica method,[127] combinations of parallel tempering, multi-canonical and multiple histogram methods,[128] and broad-histogram MC.[129,130]
c. The so-called SWARM type of MD[131] is based on the idea of combining a collection or swarm of copies of the system each with its own trajectory into a cooperative multi-copy system that searches configurational space. To build such a cooperative multi-copy system, each copy is, in addition to physical forces due to $\mathcal{V}(\mathbf{x})$, subject to (artificial) forces that drive the trajectory of each copy toward an average of the trajectories of the swarm of copies, in analogy to the fact that intelligent and efficient behaviour of a whole swarm of insects can be achieved even in the absence of any particular intelligence or forethought of the individuals. SWARM-MD is less attracted by local minima and is more likely to follow an overall energy gradient toward the global energy minimum. Other multi-copy methods can be found.[132–134]

The overviews of Figure 2 and Table 1 are meant to offer a hand when choosing a combination of search or sampling methods with various enhancement techniques that will be appropriate to model the particular system and energy function of interest, leading to an efficient calculation of the requested properties.

Biasing the Search, Sampling or Simulation

The search or sampling enhancement methods discussed in the previous section did so without a particular bias being imposed on the molecular system. However, if the particular barriers of the potential energy function or hypersurface that block access to low-energy parts of the surface can be identified, this knowledge can be put into the form of a biasing potential energy term to be added to the Hamiltonian, which will guide the trajectory in a required direction. A variety of such biased searching or sampling methods exists.

Since high-frequency motions are generally not of great interest, one may bias an MD simulation in the direction of slower modes by filtering out the high frequencies from the spectrum during a simulation.[135–137]

Another possibility is to bias the motion in an MD simulation in the direction of the principal components as obtained from the trajectory so far.[93,138–140] This bias should enhance the exploration of larger amplitude modes of the molecular system. Yet another method couples the collective modes of a system to a bath of higher temperature than the other modes.[141] This enhances the sampling along the collective modes.

Recently, a method to enhance sampling of rare events was proposed, which makes use of distance or torsional-angle restraints to overcome an energy barrier separating two metastable states, or to stabilise a transition state between the two metastable states.[142] The latter states are not subject to restraints, which allows one to determine the free energy difference between the two metastable states without the need to choose a physically realistic pathway connecting them.

Sampling or Simulation Along Pathways

Dynamical processes in biomolecular systems may occur on time scales far beyond the ones that are accessible through standard MD simulations. If these processes are intrinsically slow, i.e. require an extensive sampling of configuration space, not much can be done to speed up their simulation without destroying the dynamics of the system. If, however, these processes are rare, i.e. they do not occur often, but when occurring they are fast, there are possibilities to enhance the sampling of these rare processes. Generally they are characterised by the need to pass over a high-energy barrier separating two meta-stable states.

The oldest approach to sample transitions is to define a reaction coordinate or transition pathway and to sample along this path using a biasing potential energy term and umbrella sampling.[143] A variation using MD is so-called targeted MD.[144–146] A more sophisticated methodology is transition path sampling, which finds transition pathways for infrequent events and requires no knowledge of the transition mechanism or transition state, only the end states need be defined.[147,148] Although this method is more powerful than traditional reaction-coordinate sampling, the requirement of a proper definition of the two end states restricts its applicability. The method and its applications have been recently reviewed.[148,149] A novel extension is called transition interface sampling.[150]

Other methods to determine and sample transition pathways are the finite temperature string method, which generates a tube in configuration space between the end states, inside which conformational changes occur with high probability. This leads to an increased rate of occurrence of the rare transitions.[151,152] A minimum-energy path of a transition can be obtained through the nudged elastic band method.[153–156]

An alternative way to speed up rare events was called "hyper-MD".[157,158] It uses a biasing potential to guide the dynamics away from the end states. Yet another scheme is called coarse MD.[159]

Use of Other Than Spatial Molecular Coordinates When Searching or Sampling

The methodology discussed in the previous sections is based on the use of molecular spatial coordinates as variables which are sampled. This approach is widely used, but may not lead to effective solutions when the energy hypersurface is characterized by extremely high potential energy barriers separating different tightly packed, low energy conformations. Problems of this type are the docking of inhibitor or substrate molecules into an active site of an enzyme or the prediction of dominant side-chain conformations of amino acid residues in mutated proteins. For such cases one may use a rather different search and sampling technique, in which not only the molecular coordinates $\mathbf{x}$ serve as variables, but also the Boltzmann probability P_α of occurrence of a molecular conformation x_α,

$$P_\alpha = \frac{e^{-\mathcal{V}(\mathbf{x}_\alpha)/\mathbf{k_B T}}}{\sum_{\alpha'} e^{-\mathcal{V}(\mathbf{x}_{\alpha'})/\mathbf{k_B T}}}. \quad (11)$$

The computational problem is now to minimize the average potential energy

$$\langle E \rangle = \sum_\alpha P_\alpha \mathcal{V}(\mathbf{x}_\alpha) \quad (12)$$

subject to condition (11). That is, one wishes to find a Boltzmann distributed ensemble of configurations for a very high-dimensional and complex interaction function $\mathcal{V}(\mathbf{x})$. Since the average energy $< E >$ in eq. (12) depends on both, conformational coordinates $\mathbf{x}_\alpha$ and conformational probabilities P_α, four types of search or optimization algorithms to obtain a set of $(\mathbf{x}_\alpha, \mathbf{P}_\alpha)$ values that represent a Boltzmann ensemble may be distinguished.[59]

1. Conformational coordinates $\mathbf{x}_\alpha$ are treated as variables, probabilities P_α implicitly satisfy eq. (11). This is the classical conformational search problem as discussed in the previous sections, in which the molecular coordinates $\mathbf{x}_\alpha$ are changed according to classical (constant T) mechanics (MD) or using a Markov probability chain (MC) such that the probabilities P_α automatically satisfy eq. (11).
2. Multiple conformations $\mathbf{x}_\alpha$ are used simultaneously, but kept fixed $(\alpha_1, \alpha_2, \alpha_3, \ldots)$, probabilities P_α are treated as variables, which follow from eq. (11). This approach works when the relevant conformations $\mathbf{x}_{\alpha_1}, \mathbf{x}_{\alpha_2}, \ldots$ can be easily identified *a priori*.[160,161]
3. Multiple conformations $\mathbf{x}_\alpha$ are used simultaneously as variables that change according to classical equations of motion, probabilities P_α are treated as parameters that adiabatically follow the variation of $\mathbf{x}_\alpha$ according to eq. (11). This approach[59] has been demonstrated using a cyclic peptide.[161]
4. Multiple conformations $\mathbf{x}_\alpha$ and probabilities P_α are used simultaneously as variables that change according to classical equations of motion. This is a generalization of the previous approach.[161] The Boltzmann relation (11) can be imposed on the variables $(\mathbf{x}_\alpha, \mathbf{P}_\alpha)$ either in the form of a penalty function for P_α which is added to the standard interaction function $\mathcal{V}(\mathbf{x}_\alpha)$, or in the form of a constraint to P_α, which is to be satisfied when the equations of motion for $(\mathbf{x}_\alpha, \mathbf{P}_\alpha)$ are integrated.

Discussion

An overview of the types of methods that are currently used in biomolecular modeling to search or sample or dynamically move through the configurational space of a molecular system was given. Since in general the configurational space is too large to be completely searched or sampled, the various methods (Section III) and techniques aim at reducing the size of the problem (Section II), or at using particular algorithms to enhance efficiency (Section IV), or at transforming the problem into a more tractable one for which solutions can be found that are good approximations to solutions of the original problem (Section V). Generally, saving computational effort by the techniques presented has its price: the accuracy of the generated ensemble or trajectory is decreased depending on the search or sampling enhancement technique used or depending on the type of degrees of freedom that are omitted from the calculation. Whether such a loss in accuracy of particular properties is acceptable depends on the goals of a modeling study. In this respect one may distinguish four different degrees of distortion of the correct result induced by search or sampling (enhancement) techniques.

1. Techniques that preserve the correct dynamics of the system (no distortion).
2. Techniques that distort the dynamics, but generate a correct Boltzmann ensemble.
3. Techniques that distort dynamics and ensemble; they only generate an arbitrary collection of molecular configurations.
4. Techniques that yield neither dynamics nor an ensemble or arbitrary set of molecular configurations, but only one molecular configuration.

For example, when relative free energies of binding or complexation are to be obtained, correct dynamics is not required, only a proper ensemble.[162]

If the biomolecular modeling problem can be formulated in terms of particular conformational states, the search and sampling problem is reduced to pathways connecting such (end) states (Section VII) and efficiency may be enhanced using biasing techniques (Section VI). For example, in free energy calculations unphysical pathways may be used to obtain the relative free energies of two end states.[162]

The bulk of the methods and techniques that were discussed are based upon variation of spatial molecular coordinates. Yet methods that use other molecular coordinates have been proposed and found some use (Section VIII).

Of the many search and sampling methods and enhancement techniques reviewed a few are very effective: use of soft-core atoms, local-elevation simulation and its derivatives, replica-exchange simulation and generalized-ensemble methods. When end states are known, transition path sampling is a powerful method. For other reviews of search and sampling methodology we refer to refs. 26, 59, 75, 148, and 163–165. The present one is meant to support the practical biomolecular modeler when choosing a combination of methods and tricks that will be particularly suited to the specific problem, i.e. molecular system and properties to be computed, of interest.

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